European Journal of Chemistry 4 (1) (2013) 58-60

European Journal of Chemistry
Journal homepage: www.eurjchem.com

# Validation of analysis method for determining ketoprofen concentration in pharmaceutical dosage form using high performance liquid chromatography

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ARTICLE INFORMATION

Received: 14 September 2012
Received in revised form: 28 October 2012
Accepted: 02 November 2012
Online: 31 March 2013

KEYWORDS

Conformity
Optimization
Ketoprofen
Phosphate buffer
Validation method
High performance liquid chromatography

ABSTRACT

The study was conducted with the purpose to develop and validate a high performance liquid chromatography method with UV detector to determine ketoprofen content in tablet preparation using ethanol-phosphate buffer (pH = 6.0, 80:20, *v:v*). The method was validated toward parameters of accuracy, precision, linearity, selectivity, LOD and LOQ. The results obtained fulfill the validation requirement of the ketoprofen tablet in the form of LOD = 0.5302 ppm and LOQ = 1.7676 ppm.

## 1. Introduction

Ketoprofen is an non-steroidal anti-inflammation drug, used in this study by considering various aspects [1,2], namely solubility, weak acid compound and easily dissolve, in ethanol, chloroform and ether [3]. Ketoprofen dissolution can also be increased by adding 0.2 M phosphate buffer with pH = 5-7 up to 3-105 times compared to its solubility in water [4].

High performance liquid chromatograph (HPLC) is often used to validate method of analysis by first optimizing various parameters [5-10]. The optimization is important for the effectiveness and efficiency of instrument. The validation parameters reported in this paper are accuracy, precision, selectivity, detection limit, linearity, and robustness of validated for the estimation of ketoprofen standard as well as tablet dosage form [11,12].

## 2. Experimental

HPLC instruments consist of phase isokratil type Shimadzu brand version 6.1 joined with SCL-10 AVP control system, detector UV-VIS SPD-10 AMP, pump LC 10 ADVp, oven column CTO-10 ACVP. Shim-pack column VP-ODS 250 x 4.6 mm. Filtration unit for HPLC (Shibata), ultrasonic bath (Shibata type SU-2 TH), and spectrophotometer UV-Vis (Shimadzu type 1601). Ketoprofen tablet 50 mg from the factory sample, ketoprofen standard of comparison from PT Pharos with LSA: 14901595 and gross weight: 0.051 kg. Difference: 0.001 kg, Net weight: 0.050 kg, Phosphate buffer pH = 5.6 and 7.0 with degree of pro analysis reaction, ethanol 99.99% gradient grade for liquid chromatography.

### *2.1. Preparation of Ketoprofen standard solution*

About 10 mg ketoprofen standard was weighed, then dissolved with ethanol in flask of 10 mL up to the sign limit, and homogenized to obtain concentration of ketoprofen standard solution 1000 ppm.

### *2.2. Optimization of mobile phase volume*

Ketoprofen standard solution 1000 ppm, pipetted 0.02mL was put into the flask of 10 mL, diluted with ethanol up to the sign limit, then injected as much as 25 µL into the HPLC with mix mobile phase of ethanol phosphate buffer pH = 5 (95:5, *v:v*) using speed flow 1 mL/min with 10 minutes retention time. The experiment was replicated 3 times and also the mix mobile phase of ethanol phosphate buffer (90:10, *v:v*; 85:15, *v:v* and 80:20, *v:v*), then the mix mobile phase was chosen to provide the best separation based on HETP and number of plats theory (N).

### *2.3. Test of system conformity*

Ketoprofen standard solution 1000 ppm was pipetted 0.2 mL and put into the flask of 10 mL and diluted with ethanol up to the sign limit, homogenized to obtain ketoprofen standard concentration each 20 ppm with three replications made. Then each replication was injected 25 mL into HPLC with optimum mix mobile phase, optimum pH phosphate buffer, the detector was arranged at the selected wave length and used speed flow 1 mL/min with 10 minutes retention time to obtain chromatogram for each concentration to be used in determining the repetition of standard solution change stated in relative standard deviation.


ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online) 
DOI:10.5155/eurjchem.4.1.58-60.678

**Table 1**. Accuracy of test result method of standard additions of ketoprofen in the sample (concentration 80%) in ethanol-dafarfosfat mobile phase pH = 6 (80:20, *v*:*v*) (n=5).

| No | Tablets (mg) | Standard (mg)($C_A$) | Sample (mg)($C_A$) | Total Ketoprofen (mg) | Rt (minutes) | Area (mAU*S) | Content ($C_F$) | Recovery (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 129 | 13 | 28.165 | 41.165 | 6.517 | 16176097 | 41.237 | 100.553 |
| 2 | 129 | 12 | 28.165 | 40.165 | 6.342 | 15709253 | 40.005 | 98.666 |
| 3 | 128 | 12 | 27.947 | 39.947 | 6.358 | 15716001 | 40.023 | 100.633 |
| 4 | 130 | 14 | 28.384 | 42.384 | 6.392 | 16653791 | 42.497 | 100.807 |
| 5 | 130 | 14 | 28.384 | 42.384 | 6.250 | 16569073 | 42.273 | 99.207 |
| Average | | | | | | | | 99.973 |
| Standard deviation | | | | | | | | 0.752 |
| % Average standard deviation | | | | | | | | 0.867 |

**Table 2.** Repeability of test result assay of ketoprofen (a concentration of 20 ppm) in ethanol-dafarfosfat pH = 6 (80:20, *v*:*v*) (n=5).

| No | Area (mAU*S) | Peak Height (mAU) |
|---|---|---|
| 1 | 5354423 | 374391 |
| 2 | 5374777 | 375422 |
| 3 | 5360187 | 372357 |
| 4 | 5302888 | 374121 |
| 5 | 5150702 | 364068 |
| Average | 5322948 | 307021.8 |
| Standard deviation | 96702.570 | 4608.250 |
| % Average standard deviation | 1.739 | 1.238 |

**Table 3.** Repeability of test result assay of ketoprofen (a concentration of 100 ppm) in ethanol-dafarfosfat pH = 6 (80:20, *v*:*v*) (n=5).

| No | Area (mAU*S) | Peak Height (mAU) |
|---|---|---|
| 1 | 23835932 | 1610843 |
| 2 | 23703430 | 1630495 |
| 3 | 23252063 | 1635901 |
| 4 | 24447081 | 1650709 |
| 5 | 23418695 | 1692778 |
| Average | 23907026.8 | 1644145 |
| Standard deviation | 477493.809 | 30706.53 |
| % Average standard deviation | 1.944 | 1.867 |

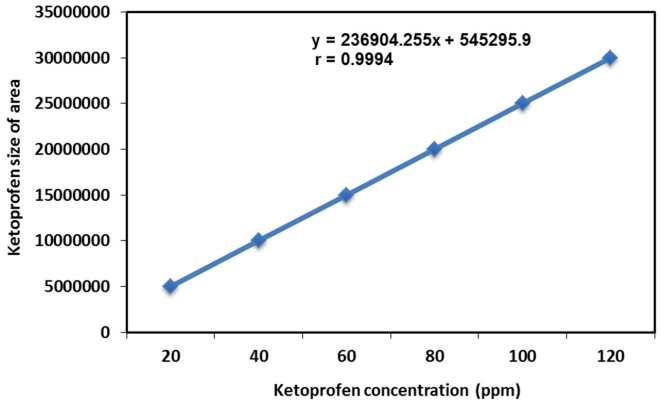


**Figure 1.** Calibration curve by Ketoprofen by HPLC.

### *2.4. Linearity*

In order to determine linearity between ketoprofen concentration and size of area, the solution series were prepared: 20, 40, 60, 80 and 100 ppm from ketoprofen standard solution 1000 ppm. The result can provide equation of regression line $y$ = 236904.255 $x$ + 545295.9. The calculation of ketoprofen content used the equation line. The correlation value indicates that there is a good correlation between ketoprofen concentration and the size of area (Figure 1).

### *2.5. Accuracy*

The method applied was the addition of sample standard and then analyzed. The difference of result was compared with added standard content as percentage of re-planning. The accuracy method is said to be good if the range of regain 98-102% (Table 1).

The recovery according to data was arranged with concentration 80% each was replicated 5 times and the average obtained is 99.973-50.752 % with range 98.686-100.807 % and value of averagedeviation standard 0.867.

### *2.6. Precision*

The test was done by examining low concentration as much as 20 ppm and high concentration 100 ppm each was replicated 5 times in method range. By using the result of standard dissolution in different concentration, the size of area can be known and peak height provided by HPLC shows that the method used to determine ketoprofen content is precise since the Average Deviation Standard of reexamination < 20% as can be seen in Table 2 and 3.

**Table 4**. Test result of content uniformity of Ketoprofen inthe factory sample 50 mg (n=10).

| No | Tablets (mg) | Sample (mg) | Rt (minutes) | Area (mAU*S) | Content of Ketoprofen each tablet | Content of Ketoprofen (%) |
|---|---|---|---|---|---|---|
| 1 | 229 | 50 | 6.383 | 18254098 | 46.719 | 93.438 |
| 2 | 231 | 50 | 6.433 | 19217996 | 49.262 | 98.524 |
| 3 | 228 | 50 | 6.533 | 20148247 | 51.715 | 103.431 |
| 4 | 239 | 50 | 6.533 | 19148247 | 49.078 | 98.156 |
| 5 | 224 | 50 | 6.417 | 19956240 | 51.209 | 102.419 |
| 6 | 231 | 50 | 6.650 | 20342563 | 52.229 | 104.458 |
| 7 | 228 | 50 | 6.667 | 18245556 | 46.696 | 93.393 |
| 8 | 230 | 50 | 6.683 | 20229079 | 51.929 | 103.859 |
| 9 | 229 | 50 | 6.342 | 18380439 | 47.052 | 94.105 |
| 10 | 230 | 50 | 6.308 | 18221926 | 46.634 | 93.268 |
| Average | | | | | 49.252 | 98.5051 |
| Standard deviation | | | | | 2.371 | 4.742 |
| % Average standard deviation | | | | | 4.814 | 4.814 |

**Table 5.** Test result of content uniformity of Ketoprofen in the factory sample 50 mg (n=6).

| No | Tablets (mg) | Sample (mg) | Rt (minutes) | Area (mAU*S) | Content of Ketoprofen each tablet | Content of Ketoprofen (%) |
|---|---|---|---|---|---|---|
| 1 | 229 | 50 | 6.700 | 20196206 | 51.842 | 103.684 |
| 2 | 231 | 50 | 6.383 | 19870053 | 50.982 | 101.964 |
| 3 | 230 | 50 | 6.675 | 20047037 | 51.449 | 102.898 |
| 4 | 230 | 50 | 6.342 | 20093247 | 51.571 | 103.142 |
| 5 | 231 | 50 | 6.325 | 19535645 | 50.100 | 100.200 |
| 6 | 232 | 50 | 6.392 | 20593911 | 52.892 | 105.784 |
| Average | | | | | 51.427 | 102.945 |
| Standard deviation | | | | | 0.925 | 1.851 |
| % Average standard deviation | | | | | 1.798 | 1.798 |

## 3. Results and discussion

The application of content determination method in 10 tablets each contains 50 mg ketoprofen indicates the uniformity of average content 49.252 mg/tablet and ketoprofen content 98.51%(Table 4).

Based on the uniformity test of content, the content uniformity is good enough since the determination of content of each tablet, the ketoprofen content ranges from 93.29 to 104.46% with %average deviation standard = 4.814 if it is calculated with the data of peak area size. The value fulfills the general requirement of content uniformity of quality matter that is at the range 90-100% with % average deviation standard < 6%.

The result of ketoprofen content determination in tablet with six replications can be seen in Table 5.

Table 5 shows that the average ketoprofen content in sample tablet preparation with 50 mg/tablet based on chromatogram area is 57.472 ± 0.925 mg/tablet (%R.S.D. = 1.798). This result fulfills the requirement that is 90-110% of the table of good precision since it meets the acceptance criteria < 2%.

The test of accuracy includes the regaining of concentration 80% (98.666-100.807%), concentration 100% (99.073-101.795%), concentration 120% (99.325-100.379%) all meet the criteria. The test of precision provides the repetitive value less than 2% that is % R.S.D. for each concentration tested 1.739% and 1.949%.

The test of linearity provides equation line that is y = 236904.255x + 545295.9 with the value of detection limit (LOD = 0.5302 ppm and limit of quantity (LOQ= 1.7676 ppm).The procedure of determining ketoprofen content is found in tablet preparation using HPLC Shimadzu brand version 6.1 with ethanol mobile phase: phosphate pH=6 with comparison 80:20 (*v*:*v*), speed flow 1 mL/min, using Shim-Pack column VP-ODS 250 x 4.6 mm at room temperature 27 $^{o}$C and automatic column pressure.

## 4. Conclusion

Validation levels of ketoprofen in tablet dosage form using newly developed method is reliable and can be used routinely.

***Acknowledgements***

Thanks addressed to the president of the Hasanuddin University for funding this research paper. We also thank to the head of Pharmaceutical Chemistry Laboratory of Hasanuddin University for sample preparation.

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